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Development of 'naked-tufted' seed coat mutants for potential use in cotton production

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Abstract Use of chemical mutagenesis has been highly successful in most major crops. The objective of this research was to develop 'naked-tufted' seed mutants and to incorporate this genetic trait into cotton to enhance crop quality and reduce processing costs. In 1997, six commercial cultivars were treated with 2.45% v/v ethyl methane sulfonate. In 1999, three M_3 plants were identified that had partially naked seed coats. The trait was stabilized through individual plant selections from 2000 to 2004. During 2005 and 2006, the homozygous naked-tufted M_8 mutant lines were evaluated for lint yield, lint percent, fibers/seed, fibers/mm^2, fiber quality, seed oil content, ginning efficiency and yarn spinning performance. Overall, the naked-tufted seed mutants had lower lint yield, lower fibers/seed, lower lint/seed, and lower fibers/mm^2 when compared with their original fuzzy parents. The lint turnout from the mutants was similar to the fuzzy parents and the commercial cultivars. The naked-tufted seed mutants had higher seed oil percent, 6–17% lower short fiber contents, significantly reduced seed coat neps (37–42%), higher elongation and yarn tenacity than their fuzzy counterparts. Preliminary data also showed that the naked-tufted mutants required less energy to gin.

Keywords Ethyl methane sulfonate · Fiber quality · Mutagenesis · Naked seeds

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Introduction

Use of chemical mutagenesis has been highly successful in most major crops but has only recently been used in improving cotton (Auld et al. 2000). Relatively low levels of genetic variability is currently available in cotton. Chemical mutagenesis has been shown to be an effective tool to create a wide range of phenotypic variation in both diploid and tetraploid *Gossypium* populations (Auld et al. 2000; Larik et al. 1983; Hussien et al. 1982; Shattuck and Katterman 1982; Gaibullaev et al. 1976).

Several genes are known to control the presence or absence and quantity of fuzz (Endrizzi et al. 1984; Kohel 1973). The best characterized of these are the naked seed loci, N_1N_1 and n_2n_2. These mutants lack most of the lint fibers. The fuzz (short) fibers develop but eventually fall off the seeds to produce black or 'naked seeds'. The recessive gene n_2n_2 is characteristic of many of the commercial cotton varieties of the species *Gossypium barbadense*. Generally, there is substantial lint, i.e., lint percentage above 30%, in these strains. The seeds are not totally naked, but retain fuzz at least at the tip. Phenotypicaly, N_1N_1 is slightly more extreme, much less common and consistent than n_2n_2, which is more subject to changes in genotypes and environmental conditions. The most advantageous results in terms of a naked seed are produced when the strain is homozygous N_1. The presence of dominant alleles of N_1 results in a totally naked seed, even at the tip, sometimes called a fuzzless seed. The quantity of lint is extremely limited, i.e., generally between 0 and 15%, so that such a strain or variety with high lint percent would be a long and difficult process and not likely to be successful (http://www.freepatentsonline.com/5516979.html). Their similar phenotypic effects and linkage relationships in two homeologous chromosomes (each thought to be about 15 cM from the respective centromeres of chromosomes 12 and 26) suggest they are homeologous loci (Samora et al. 1994). The gene symbols, N_1 and n_2n_2 were given and assigned to linkage groups V and IX, respectively, in *G. barbadense* L. and *G. hirsutum* L. by Thadani (1923) and Harland (1929).

Historically, genes that induce linters have been strongly associated with both low lint yield and low lint percent. Turley (2002) found lint percentage of 25.6% for accessions with n_2n_2 alleles and 11.4% for N_1N_1 alleles. In another study, Turley and Kloth (2002) identified a third fuzzless seed locus (n_3) and reported on the impact of the N_1n_1, N_2 n_2, and N_3n_3 genes on lint percent in cotton. They proposed that only the genotype $n_1n_1N_2N_2N_3N_3$ would generate a normal lint percent of 40.5%. Lee et al. (2006) reported on the gene expression of the N_1N_1 genotype and its impact on fuzz development and reduced lint yield. According to them, the dominant mutation (N_1N_1) delayed fiber cell formation and reduced the number of fiber cell initials. It reduced the total number of fiber cells, resulting in sparsely distributed short fibers. The N_1N_1 mutation, they suggested disrupts temporal regulation of gene expression, leading to a defective process of fiber cell elongation and development.

Naked seeds can be ginned by roller gins which may cause less damage or tearing to the lint during ginning. The less fuzz on the seed, the more efficient the ginning process because the fuzz creates resistance to the roller gin during ginning. Therefore, upland cotton cultivars having fuzzy seeds are generally ginned by saw gins, which tend to tear the fibers, thus reducing the length and quality of the lint. The ease of removal of lint fibers from the seed is also another issue. Naked seeds generally require lower force to remove the fibers than fuzzy seeds. Greater resistance to ginning can lead not only to torn fibers, but also to broken seed coat fragments during removal of the lint. This seed coat fragmentation is minimal in naked seeds (http://www.freepatentsonline.com/5516979.html), (Triplett 1990).

The objective of this research was to develop 'naked-tufted' cotton seed mutants that will enhance crop quality and reduce processing costs.

Materials and methods

In 1997, seeds from High Plains cotton cultivars, Atlas (PVP# 9200188), Tejas (PVP# 9500252), SC 9023 (PVP# 9500237), Sphinx (PVP# 7200045), Explorer (Associated Farmers Delinting, Littlefield, TX), Holland 338 (Holland Cottonseed, Big Spring, TX), and Rocket (Associated Farmers Delinting, Littlefield, TX), were treated with 2.45% v/v ethyl methane sulfonate (EMS). The seeds were imbibed in aerated distilled water for 16 h and rinsed with distilled water and treated with EMS for 2 h. The seeds were thoroughly rinsed with distilled water and immediately hand planted in the field. During 1997 (M_1) and 1998 (M_2), one boll/plant was harvested in bulk to form the next generation and to reduce the mutation load.

In 1999, three M_3 plants from Atlas, Tejas, and SC 9023 with partially naked seed coats were identified (Fig. 1). From 2000 to 2003, individual plant selections from the three M_3 naked-tufted seed coat mutants were made to stabilize this trait. In 2004, 2006 and 2007 the homozygous naked-tufted M_8 mutant lines were evaluated with two commercial cultivars (FiberMax 958 and 989), and three parental

Fig. 1 Reduced linters phenotype in cotton (naked seed)

lines (Atlas, SC 9023, and Tejas) for the impact of the naked-tufted phenotype on lint yield, lint percent, fibers/seed, fibers/mm^2, fiber quality, seed oil content, ginning efficiency and yarn spinning performance at Lubbock, Texas. Plots were planted under drip irrigation and were 9.12 m × 1.0 m rows. A Randomized Complete Design with four replications was used. Sixty-seven kg/ha actual nitrogen was applied as urea (32:0:0) at first bloom. Plots were harvested with a stripper and ginned with a 20-saw gin.

Fibers from stripper harvest were analyzed at the Fiber and Biopolymer Research Institute, Texas Tech University, using High Volume Instruments (HVI), Advanced Fiber Information Systems (AFIS), and standard yarn quality analyses. Indirect oil content analysis was carried out using the Nuclear Magnetic Resonance (NMR) Spectroscopy. The NMR technology measures the resonance energy absorbed by hydrogen atoms in the liquid state of the sample. The NMR method gives very accurate and precise results when calibrated carefully. To determine fatty acid composition, nonvolatile fatty acids were chemically converted to the correspondingly volatile methyl esters. The resulting volatile mixtures were then analyzed by gas chromatography. Lint/seed was measured from 30 g seed cotton manually ginned. The weight of lint from the 30 g samples was divided by the number of seeds from the 30 g to calculate lint/seed. To calculate fibers/seed and fibers/mm^2, seed were first scanned for total surface area with Winseedle scanner (http://www.regent.qc.ca/products/needle/NEEDLE.html). The mean length by number and fineness data from AFIS from the 30 g sample was then used to calculate the number of fibers/seed by dividing the mean surface area to obtain the number of fibers/mm^2. Seed nakedness was visually estimated using a scale of 0–100% (0 being fully fuzzy and 100 being fully naked). The SAS software package (SAS Institute Inc., SAS Circle, Cary, NC) was used to analyze all data. Pearson's Correlation Coefficients were used to test for associations between seed nakedness, lint yield, lint turnout, short fiber contents, neps (fiber entanglements) and energy requirements for ginning.

Results and discussion

These mutants appear to reduce or eliminate the occurrence of fuzz or linters which are short fibers tightly attached to the seed coat. These mutants appear to be phenotypicaly similar to the "naked-tufted" mutant initially described by Endrizzi and Ray (1991). Lint yield and lint percent for three naked seed mutants (Atlas-NS-129-2-1-1-1, SC 9023-NS-57-13-2-1, and Tejas-NS-28-13-3-1-1) and their original parents (Atlas, SC 9023, and Tejas) plus two FiberMax check cultivars (FiberMax 989 and FiberMax 858) are given in Table 1. Overall, the naked-tufted seed mutants had

Table 1 Lint yield, turnout and seed nakedness for some naked seed mutants, their original parents and check cultivars

Cultivar/Mutant	Lint yield (kg/ha)				Turnout (%)					Seed nakedness
	2004	2006	2007	Mean	2006 (LBB)	2007 (LBB)[a]	2007 (LBB)[b]	2007 (Col.St)[c]	Mean	
Atlas	1,549	1,533	1,773	1,618	40	39	44	37	40	0.00
Atlas-NS-129-2-1-1-1	1,201	1,332	1,707	1,413	38	41	41	33	38	0.36
SC 9023	1,373	1,508	1,650	1,510	40	37	40	36	38	0.00
SC 9023-NS-57-13-2-1	1,000	1,539	1,389	1,309	39	37	39	34	37	0.37
Tejas	1,503	1,494	1,714	1,570	41	39	42	37	40	0.00
Tejas-NS-28-13-3-1-1	1,181	1,352	1,560	1,364	43	39	40	37	40	0.36
FiberMax 989	962	1,406	–	1,184	43	–	44	–	44	0.00
FiberMax 958	1,138	1,659	–	1,399	43	–	47	41	44	0.00
CV (%)	18.6	10.8	12		4.8	5.3	4.6	5.0		8.1
LSD (0.05)	375	202	265		2.0	3.4	3.4	8.4		0.05

[a] Lubbock, TX, Location 1

[b] Lubbock, TX, Location 2

[c] College Station, TX

lower lint yield than their fuzzy original cultivars. This could be because of the lower lint/seed, lower fibers/seed and the resulting fibers/mm^2 (Table 2). Contrary to earlier reports (Turley 2002; Turley and Kloth 2002; Lee et al. 2006) the gin turnout of the naked seed lines ranged from 37 to 40%, which is very similar to the gin

Table 2 Lint per seed, fibers per seed, and fibers per square mm for some naked seed mutants, their original fuzzy parents and check cultivars

Cultivar/Mutant	Lint per seed (mg)			Fibers per seed (No.)			Fibers per square mm		
	2006	2007	Mean	2006	2007	Mean	2006	2007	Mean
Atlas	68.6	70.3	69.5	17,767	19,462	18,615	181	192	187
Atlas-NS-129-2-1-1-1	57.0	63.3	60.2	15,206	19,888	17,547	157	206	182
SC 9023	66.9	65.7	66.3	17,013	17,706	17,360	173	168	171
SC 9023-NS-57-13-2-1	59.3	56.7	58.0	15,652	16,416	16,034	161	165	163
Tejas	69.4	74.7	72.1	18,636	20,989	19,813	194	193	194
Tejas-NS-28-13-3-1-1	72.8	75.3	74.1	18,893	20,032	19,463	181	177	179
FiberMax 989	76.2	78.0	77.1	19,834	19,920	19,877	210	201	206
FiberMax 958	77.6	87.0	82.3	19,223	21,432	20,328	196	209	203
CV (%)	7.0	6.5		7.2	10.0		7.2	9.7	
LSD (0.05)	3.8	8.2		1,019	3,420		10.3	32	

Table 3 Oil content and fatty acid composition for some naked seed mutants, their original fuzzy parents and check cultivars

Cultivar/Mutant	Oil content (%)			Fatty acid composition[a]			
	2006	2007	Mean	Palmitic (16:0)	Stearic (18:0)	Oleic (18:1)	Linoleic (18:2)
Atlas	15.4	20.1	17.8	24.5	2.7	19.6	49.6
Atlas-NS-129-2-1-1-1	17.4	20.3	18.9	22.5	2.8	19.3	54.3
SC 9023	14.8	18.4	16.6	25.0	2.7	17.4	51.5
SC 9023-NS-57-13-2-1	17.1	21.1	19.1	21.1	2.8	19.3	53.2
Tejas	15.9	20.7	18.3	23.6	2.7	19.8	50.8
Tejas-NS-28-13-3-1-1	16.9	19.9	18.4	24.1	2.9	18.1	51.7
FiberMax 989	15.7	20.7	18.2	24.0	2.7	17.4	52.8
FiberMax 958	15.8	20.5	18.2	24.9	2.7	18.7	50.5
CV (%)	2.4	5.2		4.8	6.5	5.6	2.5
LSD (0.05)	0.8	1.8		2.2	0.4	2.1	2.7

[a] Normal cotton on the average has 22% Palmitic, 15% Oleic, 49% Linoleic, and 2.7% Stearic

Table 4 Pearson correlation coefficients for some parameters for the naked seed mutants

	Percent oil	Lint yield	SFCn[a]	SFCw	nepSize[b]	nepCnt	Average total PRWatt[c]	Average total SawWatt[d]
Nakedness	0.9270	−0.1287	−0.0574	−0.0684	−0.1923	−0.1523	−0.3374	−0.0179
	0.001	0.723	0.595	0.527	0.073	0.157	0.340	0.961
Turnout	−0.7384	0.4134					0.5892	−0.2220
	0.015	0.001					0.073	0.538

[a] SFC = Short fiber content. Percent of fibers shorter than ½ inch

[b] neps = A small knot of entangled fibers that will not straighten to a parallel position during processing

[c] Total PRWatt = The average total for the power roll located in the seed box

[d] Total SawWatt = The average total watts used by the saw during ginning

Table 5 AFIS (Advanced Information System) data for some naked seed mutants, their original fuzzy parents and check cultivars

Cultivar/Mutant	Short fiber counts (w) (%)						Seed Coat Nep (count) (gm)						Upper quartile length (mm)					
	2006 Lbb[a]	2007 Lbb[b]	2007 Lbb[c]	2007 Lbb[d]	2007 Col.St[e]	Mean	2006 Lbb	2007 Lbb1	2007 Lbb2	2007 Lbb3	2007 Col.St	Mean	2006 Lbb	2007 Lbb1	2007 Lbb2	2007 Lbb3	2007 Col.St	Mean
Atlas	5.8	9.6	8.2	7.6	7.3	7.7	25.3	9.3	5.7	26.7	12.3	15.9	30.7	30.2	30.5	30.7	29.0	30.2
Atlas-NS-129-2-1-1-1	4.9	7.1	6.9	5.8	7.4	6.4	11.5	9.3	5.3	12.7	7.3	9.2	32.5	30.7	31.0	31.8	30.2	31.2
SC 9023	6.2	8.9	6.5	6.0	6.3	6.8	26.8	10.3	17.0	18.0	8.3	16.1	32.8	32.3	31.5	33.3	29.7	32.0
SC 9023-NS-57-13-2-1	5.0	8.7	7.0	5.9	5.6	6.4	13.1	11.3	9.7	11.3	5.0	10.1	30.5	29.2	29.2	32.8	29.2	30.2
Tejas	6.4	7.3	7.1	6.2	6.4	6.7	25.9	14.0	9.7	12.3	9.7	14.3	29.5	30.5	29.5	31.5	29.0	30.0
Tejas-NS-28-13-3-1-1	4.5	6.6	7.2	5.7	5.8	6.0	11.6	5.7	12.7	8.0	7.0	9.0	31.2	30.2	30.7	31.5	30.0	30.7
FiberMax 989	5.1	7.3	–	5.2	–	5.9	18.6	6.7	–	8.3	–	11.2	32.8	31.5	–	32.8	–	32.3
FiberMax 958	4.8	7.4	6.5	4.6	6.2	5.9	21.2	5.0	7.0	15.3	5.7	10.8	32.8	31.5	32.0	32.5	31.5	31.8
CV (%)	11.2	7.7	12.8	23.4	9.1		30.5	36.6	52.6	61.1	37.4		1.8	1.9	1.1	2.6	1.8	
LSD (0.05)	0.7	1.1	1.6	2.4	1.0		4.4	5.7	9.0	15.1	5.3		0.02	0.04	0.02	0.06	0.04	

[a] Lubbock, TX, Location 1

[b] Lubbock, TX, Location 2

[c] Lubbock, TX, Location 3

[d] Lubbock, TX, Location 4

[e] College Station, TX

turnout from the fuzzy parents (38–40%). Turley and Kloth (2002) suggested that the only genotype that would generate a normal lint percent of 40.5% is $n_1n_1N_2N_2N_3N_3$.

In 2006, all three mutants had significantly higher oil percent than their original fuzzy parents. In 2007, two mutants had higher oil percent but the result was significantly higher only for the mutant SC 9023-NS-57-13-2-1 (Table 3). No significant differences were observed in fatty acid composition between the naked seed lines and their original fuzzy parents. Seed nakedness was highly and positively correlated with percent oil (0.93**, $N = 10$). It was also negatively correlated with nep size, nep count and short fiber content. These correlations, however, were not significant (Table 4). AFIS (Advanced Information System) analyses indicated 6–17% less short fiber counts in the naked seed lines as compared to the fuzzy lines. Significantly reduced seed coat neps (37–42%) in the naked seed lines was also observed. Triplett (1990) also reported that a naked seed mutant (N_1) had 40–60 times fewer neps than the fuzzy Texas Marker-1 control line.The upper quartile length of the naked seed lines, were comparable or better than the corresponding values of their fuzzy counterparts (Table 5).

Yarn quality for ring spun yarn 40ne for the naked seed mutants, their original parents and check cultivars are given in Table 6. The naked seed mutants had higher count strength product (a measure of yarn strength), elongation, tenacity, and work (a measure of the energy to break the yarn), exhibiting their superiority in these parameters. Lower values for CV, thin and thick places, N200, and hair in the naked seed mutants further demonstrated the higher yarn quality of the naked seed materials.

Preliminary data obtained from the USDA ginning lab at Lubbock, TX indicated the superior ginning efficiency demonstrated by the naked seed mutants as compared with two fuzzy commercial cultivars, FiberMax 958 and FiberMax 989. One of the naked seed mutants required 349 watts to gin a pound of seed cotton as compared to 377 watts for FiberMax 958 (Fig. 2). As observed in Table 4, as seed nakedness increased, the average total PRWatt (the average total for the power roll located in the seed box) and SawWatt (the average total watts used by the saw during ginning) decreased. This decrease, however, is not statistically significant. A large scale ginning of these materials, probably on a commercial scale, should be conducted before a valid inference on ginning efficiency can be made.

Conclusion

The naked-tufted seed cotton mutants developed through chemical mutagenesis had lower lint yield, similar turnout, significantly increased oil content

Table 6 Yarn Quality for ring spun yarn 40Ne for some naked seed mutants, their original fuzzy parents and check cultivars

Cultivar/Mutant	Count Strength Product[a]	Elongation[b]	Tenacity[c]- (cN/tex)	Work[d]	CV[e] (%)	Thin Places[f]	Thick Places[f]	N200	Hair[g]
Atlas	2,140	5.9	13.0	317	20.0	158	958	642	5.0
Atlas-NS-129-2-1-1-1	2,228	6.1	13.9	339	19.2	125	860	526	4.5
SC 9023	2,240	6.1	13.8	340	18.8	101	735	466	4.4
SC 9023-NS-57-13-2-1	2,312	6.3	13.8	347	18.0	68	582	375	4.3
Tejas	2,192	5.9	13.7	318	19.3	144	852	535	4.5
Tejas-NS-28-13-3-1-1	2,329	6.1	14.2	339	18.9	109	785	511	4.5

[a] A measure of yarn strength. Varies from a low of 1,500 to a high of about 3,000

[b] The amount of extension or stretch of a bundle of fibers during a tension test

[C] The strength of a single strand of yarn. The force required to break a yarn

[d] A measure of the energy required to break the yarn (combines both strength and elongation)

[e] Coefficient of variation of yarn mass

[f] Thin and thick places: long yarn defects

[g] A measure of the amount of fiber ends and loops protruding from the yarn core

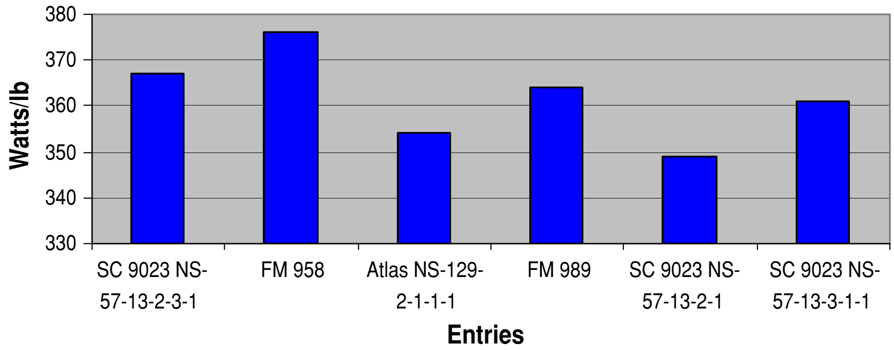

Fig. 2 Total Watts/lb of seed cotton (Source Gregg Holt–USDA)

($R = 0.68^{***}$, $df = 86$), low energy ginning and oil extraction than their original fuzzy parents. Furthermore, these mutants had improved delinting efficiency, significantly reduced seed coat neps ($R = 0.47^{***}$, $df = 86$) and reduced short fiber content. In the future, continued selections within crosses of our mutant lines with germplasm lines with extremely high rates of fiber initiation should allow us to identify lines with sufficient lint yields and enhanced fiber quality to commercialize this unique trait.

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